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Review Article

New perspective towards therapeutic regimen against SARS-CoV-2 infection

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ABSTRACT

The ongoing enormous loss of human life owing to Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2), has led to a global crisis ranging from the collapse of health – care systems to socio-economic instability. As SARS-CoV-2 is a novel virus, very little information is available from researchers and therefore, a rigorous effort is required to decode its pathogenicity. There are no licenced treatment options available for treating SARS-CoV-2 infections and the development of a new antiviral drug targeting coronavirus cannot happen soon. Consequently, drug repurposing is a promising solution for combating the present pandemic. In this review, we have thoroughly discussed all the proteins encoded by the SARS-CoV-2 genome; their importance in pathogenicity and their potential role in drug discovery. Also, the budding threat of co-infections by other pathogenic microbes has been highlighted. Furthermore, the advances made in the medicinal field for the treatment and prevention of this viral infection is explained. Altogether, this review will provide some insightful discussions about this infectious disease and will meet certain of the knowledge gaps which exist by presenting an exhaustive and extensive scientific report on the ongoing mission for COVID-19 drug discovery.

Contents

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Introduction

Coronaviruses (CoVs) are a large family of RNA viruses, belongs to the family Coronaviridae and order Nidovirales [1]. These viruses are protected by a membrane envelope and possess a large RNA genome (26–32 kb), which is single-stranded, with positive (mRNA) polarity (ssRNA+) and a nucleocapsid of helical symmetry [2]. The club-shaped glycoprotein spikes projecting from the virus surface give an electron micrograph image that resembles the solar corona; hence its name "coronavirus" [3]. Both human beings and animals are targeted groups of Covs; however, bats have been found to host the largest variety of Covs [4]. There are four classes of coronaviruses, namely: alpha, beta, gamma, and delta. The beta-Covs class harbours Severe Acute Respiratory Syndrome (SARS) virus (SARS-CoV), Middle East Respiratory Syndrome (MERS) virus (MERS-CoV), and the COVID-19 causative agent SARS-CoV-2. Coronaviruses attack the host's lower respiratory system, resulting in viral pneumonia. However, SARS-CoV-2 may also affect different organ systems including the central nervous system, leading to multiple organ failure [5,6]. SARS-CoV-2 has also been identified as more contagious than other betaCovs [7].

The first worldwide coronavirus outbreak, recorded between 2002–2004, was caused by SARS-CoV. The disease caused around 8098 SARS positive cases with a highly infectious mortal form of pneumonia and 774 reported deaths [8]. The second coronavirus outbreak was caused by MERS-CoV towards the end of 2012, which affected around 27 countries, resulting in 2494 positive cases and 858 reported deaths [9]. The disease symptoms ranged from mild to acute respiratory distress syndrome [9].

Coronavirus disease 2019 (COVID-19), reported in late 2019, is the third ruthless coronavirus outburst; caused by SARS-CoV-2. Symptoms range from mild (fever, cough and shortness of breath) to acute (severe pneumonia resulting in multi-organ failure) [10]. As reported by the World Health Organization (WHO), as of 09 September 2020 there were a total 27,417,497 confirmed cases and 894,241 mortalities globally [11]. There was increasing concern due to the fast spread of this disease, and therefore a global emergency was declared by WHO on January 31, 2020, and on March 11, 2020 the disease was documented as a pandemic. The world is desperate to uncover ways that can control the spread of novel coronaviruses and to find successful treatment options. In search of effective treatments or vaccines, more than 200 clinical trials for COVID-19 are either being conducted, or patient recruitment is in process [12]. However, every day new studies are being added, as the number of cases is increasing multi-fold globally. The treatment options currently being explored vary from decade-old malaria drugs to unsuccessful Ebola treatments, to repurposing flu drugs. An antiviral drug named EIDD-2801 has been claimed by scientists to combat SARS-CoV-2 in a better way than remdesivir [13]. Another antiviral drug, favipiravir or avigan, used against influenza in Japan, was also found effective against SARS-CoV-2 infections [14]. Malaria treatments options, namely, chloroquine and hydroxychloroquine were initially reported to be an effective option against COVID-19 [15]; however, the results from clinical trial (NCT04332991) [16] and prophylaxis data recorded by Boulware and coworkers [17] were not promising. Additionally, the withdrawal of chloroquine and hydroxychloroquine from chief investigations denotes the termination of attempts to repurpose these drugs for combating SARS-CoV-2 infection [18]. Remdesivir, an unsuccessful Ebola drug, has also shown potential and is being repurposed for SARS-CoV-2 infections; however, additional clinical trials are still ongoing to ensure the effectiveness of this drug in COVID-19 patients. Despite all this, a standard treatment for SARS-CoV-2 infections is still lacking and increasing incidences of asymptomatic infections, excessive transmissibility, and a long incubation period have made COVID-19 a competent and challenging pathogen which is very difficult to contain.

Most importantly, the occurrence of microbial co-infections in COVID-19 patients complicates the situation by increasing the hitches in management of diagnosis of and prognosis for SARS-CoV-2. Therefore, clinicians cannot ignore the high chance and risk of co-infections caused by all groups of microbes, bacteria, viruses and fungi among COVID-19 patients that may further result in serious disease symptoms and raise the mortality rate [19]. However, it cannot be denied that coinfected microorganisms can bring hope for developing new strategies against SARS-CoV-2 infection. Therefore, in the present review we have highlighted this crucial aspect to emphasise the importance of microbial coinfection in SARS-CoV-2 infection.

Aim of this review

Although there are no targeted antiviral agents available for treatment of novel SARS-CoV-2, a remarkable amount of research is in progress to find potential treatments that can save humankind and develop vaccines that can secure our future. This review aims to strengthen the intellectual foundation of ongoing research for advances in therapeutic sciences aiming at potential drugs as well as preventive vaccines for combating of SARS-CoV-2 and other coronavirus diseases. The present review provides a cumulative description of recent information on the SARS-CoV-2 structural morphology; its characteristics, related co-infections, potential drug targets and treatment options available.

Study selection

The PubMed, ScienceDirect and Scopus databases have been exhaustively searched up till September 09, 2020, using the keywords: SARS-CoV-2/coronavirus infection, including updates on SARS-CoV-2 treatments and vaccine development, challenges in coronavirus treatments, research and development on therapeutic agents and vaccines for COVID-19 and related human coronavirus

diseases and drug targets for corona viruses. Non-English articles were excluded from the study. In total 87 published articles were accessed and important cross-references were also retrieved and included in the present study. Moreover, updates from WHO, CDC, currently ongoing trials from ClinicalTrials.gov reports and other authentic sources were grouped and presented in this review article.

SARS-CoV-2

The SARS-CoV-2 genome consists of 29,811 nucleotides and is organized into a 5′ untranslated region (UTR), replicase complex (ORF1ab), spike gene (ORF2), envelope gene (ORF3a, ORF4), membrane gene (ORF5), nucleocapsid genes (ORF6, ORF7a, ORF7b, ORF8, ORF9), 3′ UTR, and other open reading frames (ORFs) yet to be characterized [20,21]. The genome encodes 29 different proteins that are divided into three main groups, namely: structural proteins (spike, envelope, membrane and nucleocapsid), non-structural proteins (NSP) and accessory proteins [22].

Structural proteins

SARS-CoV-2 encodes four main structural glycoproteins – spike (S), envelope (E), membrane (M) and nucleocapsid (N).

Spike protein (S)

S proteins are transmembrane proteins (around 150 kDa) and are projected outwards from the surface of the virus. These proteins bind the angiotensin-converting enzyme 2 (ACE2) expressed in cells of the lower respiratory tract and play a crucial role in attachment, fusion, entry, and transmission of viral particles into host cells. Furin-like protease present in the host cell cleaves S glycoproteins into two sub-units, namely the S1 part (N-terminal), responsible for virus-host receptor binding and the S2 part (C-terminal), which mediates virus-cell membrane fusion in transmitting host cells [22,23]. Therefore, the process of SARS-CoV-2 infection starts with the binding of S1- receptor-binding domain (S1-RBD) to the cell membrane receptors of the host cell, causing a structural change in the S2 part which results in fusion and the entry of the viral particle into the target cell [24,25].

Nucleocapsid protein (N)

N glycoprotein binds to the viral genome to make up nucleocapsid protein and is involved in processes such as the viral replication cycle and the host cells response to viral infections [26–28]. Although, the nucleocapsid protein N-terminal domain of this virus is structurally similar to other known Covs, but the surface electrostatic potential characteristics between them are different [29].

Membrane protein (M)

M proteins are important and most abundant proteins, which specify the outline of the viral envelope and are moreover considered as a principal organizer of CoV assembly [24]. In silico studies revealed that SARS-CoV-2 M protein has a triple helix bundle, forms a single 3-transmembrane domain (TM) and is homologous to the prokaryotic sugar-transport protein semi-SWEET. However, the advantage and role of sugar transporter-like structures in viruses are still unknown [30]. The membrane protein assists S protein during attachment and admission of the virus to the host cell. It also helps in forming a stable N protein-RNA complex and supports the completion of the viral assembly inside the virion [31].

Envelope protein (E)

E proteins are small membrane proteins (8.4−12 kDa size) that interact with M proteins to form the viral envelope and accounts for SARS-CoV-2 assembly, budding and pathogenesis [26,28]. They are found to be highly conserved among the beta coronaviruses. There are predominantly two structural domains: a hydrophobic domain and a charged cytoplasmic tail. The presence of heme agglutinin-esterase protein has been reported in some of the coronaviruses.

Non-structural proteins

The second group of proteins, non-structural proteins (NSP), play a vital role and control the assembly of the viral particle as well as its escape from the host defence system. The RNA genome that encodes these proteins is replicase complex, containing two ORFs (ORF1a and ORF1b), complete expression of which is accomplished via ribosomal frameshifting [32]. The translation of ORF1a and ORF1b produces two huge overlapping polyproteins, pp1a and pp1ab. These polyproteins are then cleaved into 16 mature smaller proteins by the papain-like protease (PLpro) and the 3-chymotrypsin-like protease (3CLpro), also known as the main protease (Mpro). From 16 proteins, the first 11 are transcribed from ORF1a and the remaining five from ORF1b [20,23]. A summary of the non-structural proteins as well as and their roles, are outlined in Table 1 below:

Accessory proteins

There are eight accessory proteins reported to date. While they are not essential for replication (suggested by in vitro studies), some of them are proved to be important for virus-host interactions. The group include ORF3a, ORF3b, ORF6, ORF7a, ORF7b, ORF8a, ORF8b, and ORF9b [51]. The details of accessory proteins reported in SARS CoV-2 and their functions are outlined in Table 2 below:

SARS-Cov-2 infection and co-infections

The individuals carrying SARS-CoV-2 are more vulnerable to co-infection with pathogens such as Aspergillus flavus, Candida species, Streptococcus pneumoniae, Staphylococcus aureus, Klebsiella pneumoniae, Haemophilus influenzae, Mycoplasma pneumoniae, Chlamydia pneumonia, Legionella pneumophila, Acinetobacter baumannii, influenza virus, coronavirus, rhinovirus/enterovirus, parainfluenza virus, metapneumovirus, and human immunodeficiency virus (HIV) [61]. Cases of SARS-CoV-2 co-infection with human metapneumovirus (hMPV), human orthopneumovirus (human respiratory syncytial virus/HRSV/RSV), Mycoplasma pneumoniae (MP), rhinovirus/enterovirus, non–SARS-CoV-2 Coronaviridae [62,63], and influenza A virus [64–66] have been reported. According to current medical literature, co-infection between SARS-CoV-2 and bacteria/fungi appear to be low. 2 out of 9 (22%) clinical studies reported bacterial co-infection in SARS-CoV-2 cases, whereas 62 out of 806 (8%) cases of bacterial/fungal co-infection were reported. These patients were put on broad-spectrum antibiotic treatment, with 72% of cases getting antibacterial therapy alone [67]. Co-infections with bacteria such as S. pneumoniae, followed by K. pneumoniae and H. influenzae were commonly reported in SARS-CoV-2 patients [68]. There are also studies reporting SARS-CoV-2 patients suffering with severe invasive pulmonary aspergillosis [69,70]. Among SARS-CoV-2 patients the proportion of bacterial co-infection was highest, followed by bacterial-viral, viral-fungal and viral-bacterial-fungal co-infections [68].

Altogether rates of co-infections in SARS-CoV-2 patients are increasing above what was previously reported. Although the number is small, the chance of co-infection is higher, which needs to be investigated in detail.

Table 1
Non-structural proteins of SARS CoV-2 and their function.

NSP	Amino acid (aa)	Function[a]	Accession number	Reference
NSP1 (leader proteins)	180 aa	Blocks host innate immune response and suppresses IFN signalling by binding to host 40S ribosome, halting translation and thereby selectively degrading host mRNA.	YP_009725297.1	[20]
NSP2	638 aa	Found conserved in another coronavirus, SARC-CoV. This protein binds with prohibitin 1(PHB1) and prohibitin 2 (PHB2) present in host, and thus is responsible for disrupting the host cell environment.	YP_009725298.1	[33]
NSP3	1945 aa	The largest protein encoded by coronavirus, it is around 200 KDa in size and a papain-like proteinase protein. This protein also eases release of NSP1, NSP2, and NSP3 from the N-terminal region. NSP3 shuts down host enzymes called PARPs, which prevent viruses from replicating.	YP_009725299.1	[34,35]
NSP4	500 aa	Accommodates transmembrane domain and interacts with NSP3 as well as host proteins and assists reorganization of SARS CoV membrane. However, both NSP4 and NSP3 are involved together for their role in viral replication.	YP_009725300.1	[36]
NSP5	306 aa	The main protease promoting cytokine expression and cleavage of viral polyprotein. SARS CoV-2 NSP5 is highly homologous to SARS NSP5 (identity, 96%; similarity, 98%).	YP_009725301.1	[37]
NSP6	290 aa	This, similarly to other CoVs, presents putative trans-membrane helices and interacts with NSP3 and NSP4. This protein is involved in generation of autophagosome from the endoplasmic reticulum and enable assembly of replicase proteins.	YP_009725302.1	[38,39]
NSP7 and NSP8	83 aa	Both NSP7 and NSP8 form a heterodimer that efficiently performs de novo initiation and primer extension. Both NSP7 and NSP8 are found conserved among 2019-nCoV, BetaCoV_RaTG, and BatSARS-like Cov.	NSP7, YP_009725303.1	[40,41]
	198 aa		NSP8, YP_009725304.1	
NSP9	113 aa	Suggested involvement in viral replication and virulence.	YP_009725305.1	[42]
NSP10	139 aa	Previous studies of SARS coronavirus demonstrate that NSP10 interacts and stimulates activity of NSP14 [S-adenosylmethionine (SAM)-dependent (guanine-N7) methyl transferase (N7-MTase)]. Additionally, NSP10 also has a crucial role in activating NSP16 (2′-O-methyltransferase).	YP_009725306.1	[43,44]
NSP11	13 aa	Function is still unknown.	YP_009725312.1	[20]
NSP12	932 aa	RNA dependent RNA polymerase (RdRp), along with cofactors NSP7 and NSP8, assists in coping viral RNA. Current studies suggest that SARS-CoV-2 NSP12 is almost identical to that of the SARS-CoV (identity, 96%; similarity, 98%).	YP_009725307.1	[45]
NSP13	601 aa	A helicase enzyme responsible for unwinding viral genome. As reported previously, the overall structure of SARS-CoV-2 NSP13 is composed of five domains giving a triangular pyramid shape similar to SARS and MERS-Nsp13.	YP_009725308.1	[46]
NSP14	527 aa	Current reports suggest it is a proofreading protein with 3' to 5' exoribonuclease (NSP14-ExoN). This activity is an important factor of both viral replication and recombination.	YP_009725309.1	[47]
NSP15	346 aa	It has been characterized as RNA uridylate-specific endoribonuclease carrying catalytic domain at C-terminal. The NSP15 protein prevents uncovering of virus within host system. This is achieved by degrading the viral polyuridine sequences.	YP_009725310.1	[48]
NSP16	298 aa	It is a N7-GpppA-specific, S-adenosyl-L-methionine (SAM)-dependent, 2'-O-MTase and is activated by binding to NSP10. NSP16-NSP10 complex cap viral mRNA transcripts protecting it from degradation by 5′-exoribonucleases, promote efficient translation and assist host innate immunity surveillance.	YP_009725311.1	[49,50]

[a] Note: The functions of NSPs are reported for SARS-CoV and are considered to be similar in SARS-CoV-2.

Potential targets for drug discovery in SARS-Cov-2

SARS-CoV-2 infection has already proven to be a devastating disease worldwide. Therefore, the most urgent timely requirement now is to advance treatment options against this disease. The strategy of drug development for tackling this global disaster can be broadly classified on the basis of specific pathways into the following categories: *(a)* blocking viral structural proteins and thereby inhibiting virus-host interaction and viral entry; *(b)* inhibiting viral RNA synthesis and replication by targeting different viral enzymes or functional proteins; *(c)* targeting viral virulence factors mediating escape from the host immune system; *(d)* targeting host-specific receptors such as Angiotensin-converting enzyme 2 (ACE2), which serves as an entry point for CoVs.

Targeting viral structural protein and its interactions

Spike protein is a crucial structural protein of CoVs and forms a trimeric structure on the surface and mediates the invasion and virulence of the virus. The S protein is also responsible for activating the host immune response toward the viral particle [71]. There-

Table 2
Accessory proteins of SARS CoV-2 and their function.

Accessory proteins	Amino acid (aa)	Function[a]	Reference
ORF3a	275 aa	Involved in formation of ion channels, virulence, infectivity and virus release	[52,20]
ORF3b	22 aa	Strong INF-1 antagonist.	[53]
ORF6	61 aa	Interacts with viral NSP8 (enhancing RNA polymerase activity) and involved in viral pathogenesis	[20]
ORF7a	121 aa	Inhibits activity of bone marrow stromal antigen 2 (BST-2) by blocking its glycosylation.	[54]
ORF7b	43 aa	Both accessory protein and structural component of the SARS virion.	[55]
ORF8	121 aa	Important for adaptation in human host following interspecies transmission and virus replicative efficiency.	[56–58]
ORF9b	97 aa	Interacts with a mitochondrial import receptor, Tom70, which acts as an essential adaptor linking MAVS to TBK1/IRF3; resulting in the activation of IRF-3	[59,60]
ORF9c	XX aa	Interacts with multiple proteins that modify IkB kinase and NF-kB signalling pathway, including NLRX1, F2RL1 and NDFIP2.	[60]
ORF10	38 aa	Function is undefined	[20]

[a] Note: The functions of accessory proteins are reported for SARS-CoV and are considered to be similar in SARS-CoV-2.

fore, targeting S proteins or specific host cell receptors is a valuable therapeutic strategy for antiviral drug development.

The receptor-binding domain (RBD) is the main target for designing drugs against SARS-CoV-2. Available literature suggested a few potential targets that hamper S1-RBD: ACE-2 binding and therefore, block the entry of SARS-CoV. The inhibitors are: OC43-HR2P (peptide derived from HCoV-OC43) showed pan-CoV fusion inhibition property [72], chloroquine (antimalarial agent, elevates endosomal pH and modifies ACE-2 binding site, thus inhibiting virus receptor binding) [73], SSAA09E2 (block the S-ACE2 binding), SSAA09E1 (blocks viral entry), SSAA09E3 (inhibits host and viral cell membrane fusion) [74], the S230 antibody nullifies various isolates of SARS-CoV [75], m396 (monoclonal antibody) competes for RBD [76], 80R and CR301 (monoclonal antibodies) are spike-specific antibodies that nullify viral infection by preventing S-ACE-2 binding [77].

The other structural proteins in SARS-CoV-2 are E protein and N protein. E protein (E-channel) owns the central function for maintaining the structural and viral pathogenicity, whereas NRBD and CRBD are the important domains of Cov N protein and they are required for an efficient host-viral interaction. Therefore, collectively, these structural proteins can be thoroughly targeted for antiviral drug discovery [78].

Targeting virus RNA synthesis and replication

Non-structural proteins are important for virus replication along with infecting the host. The most potential drug targets among them are: papain-like protease (PLpro), helicase/NTPase, 3C-like protease (3CLpro), haemagglutinin esterase and RNA-dependent RNA polymerase (RdRp), because of their strong vital role and functional enzyme active site.

C-like protease (3CLpro/Nsp5)

The 3CLpro/(Nsp5 is a homodimer protease with an active site consisting of the cys-his dyad responsible for protease activity [79]. It releases mature Nsp4-Nsp16 by cleaving Nsps present downstream at 11 sites, and facilitates production of advanced protein-mediating replication/transcription complex [80,81]. Due to important catalytic activity 3CLpro is an attractive target for developing antiviral drugs and mainly small-molecules and peptide inhibitors are used for screening [82].

An in silico study [83] indicated that antibacterial medications (oxytetracycline, demeclocycline, doxycycline and lymecycline), conivaptan (used for hyponatremia) and anti-hypertensive drugs (nicardipine and telmisartan) were inhibitors of 3CLpro. Other In silico studies suggested potential 3CLpro inhibitors among presently available drugs (aprepitant, icatibant, colistin, bepotastine, perphenazine, valrubicin, epirubicin, and caspofungin. These drugs also bind to the antiretroviral-binding site on SARS-CoV [84]. Small molecules, phthalhydrazide-substituted ketoglutamine analogs, arylboronic acids, thiophenecarboxylate and quinolinecarboxylate derivatives have been explored and proved to inhibit 3CLpro [85]. The inhibitors of HIV protease, lopinavir and ritonavir also inhibit 3CLpro [84]. Various natural products and their derivatives have been reported to show high binding affinity to 3CLpro [83].

Papain-like proteases (PLpro)

These function by slicing the N-terminus of the replicase poly protein (PP) and produce three NSPs (NSP1, NSP 2, and NSP 3) which are critical for virus replication [86]. Being vital enzymes for CoV replication and host infection, PLpro are becoming a well-accepted focus for drug advances against SARS-CoV-2. However, there is no candidate yet approved by the FDA as a drug. Zinc and its conjugates at higher doses were found to inhibit PLpro [87]. Benzodioxole [88] and a new lead compound (6577871) [89] were identified as strong inhibitors by in silico studies. Lopinavir-ritonavir combinations are also being used for treating SARS-CoV-2 infection [90]. Wu and co-workers (2020) have discussed a series of available drugs as well as natural products with strong affinity towards PLpro. The major drugs include antibacterial drugs (chloramphenicol, cefamandole and tigecycline), and antiviral drugs (ribavirin, valganciclovir and thymidine) [83].

RNA-dependent RNA polymerases (RdRp/Nsp12)

These are crucial enzymes of the replication/transcription complex and are found conserved in coronavirus. The RdRp domain of the RNA polymerase consists of a conserved motif (Ser-Asp-Asp) present at the C-terminus [91]. However, in previous beta coronavirus epidemics Nsp12-RdRp was considered as a significant drug target because inhibition of this enzyme significantly reduces toxicity and side effects in host cells [92]. Remdesivir and Ribavirin (antiviral agents) have the potential to serve as drug candidates that can block this enzyme [93]. Several other existing compounds are also presented as probable inhibitors of this enzyme, namely, itraconazole, novobiocin, chenodeoxycholic acid, cortisone, idarubicin, silybin and pancuronium bromide [83].

Helicase/NTPase

Helicase (NSP13) is a vital protein which has a critical role in the viral central dogma, with an ability to untangle double-stranded DNA and RNA in an NTP-dependent manner [94]. The SARS-Nsp13 sequence has been found conserved, and is a vital

Table 3
Drug repurposing for SARS-Cov-2.

Possible targets	Ongoing therapeutic options and their functions	Ongoing clinical trials for SARS-CoV-2
Targeting the RdRp	Remdesivir (GS-5734) [115] commonly known as Veklury; broad-spectrum antiviral drug against SARS-CoV, MERS-CoV, and various other RNA viruses.	NCT04252664, NCT04257656, NCT04252664, NCT04292899, NCT04292730, NCT04302766, NCT04323761, NCT04280705, NCT04321616, NCT04315948, NCT04314817 and NCT04349410
	Favipiravir (T-705) [117]; antiviral drug used to treat broad range of RNA viruses.	ChiCTR2000029600, NCT04358549, NCT04346628, NCT04310228, NCT04349241, NCT04336904, NCT04319900, NCT04359615, NCT04333589, NCT04303299, NCT04351295, NCT04356495 and NCT04345419
	Galidesivir (BCX4430) [118]; broad-spectrum antiviral drug.	NCT03891420
	β-D-N4-hydroxycytidine/NHC/EIDD-1931; strong inhibitory effect against MERS-CoV, SARS-CoV, and SARS-CoV-2 [119].	Data not available
	Ribavirin [118]; broad-spectrum antiviral, primarily used for treatment of hepatitis C.	NCT04356677
Inhibiting the viral protease	Ivermectin [120] (Stromectol/Soolantra cream); drug used to treat parasitic infections.	NCT04343092
	Lopinavir-Ritonavir [121]; used for treatment and prevention of HIV/AIDS.	NCT04252885 and ChiCTR2000029308
	Darunavir and Cobicistat [119, 122]; antiretroviral drug against HIV/AIDS	NCT04252274, NTC04303299 and NCT04366089
Blocking virus cell entry	Recombinant human angiotensin-converting enzyme 2 (RhACE2 APN01) [123]. Used for treating cancer and related problems.	NCT04287686 and NCT04335136
	Arbidol (Umifenovir) [124]. Used for the treatment of influenza and hepatitis C virus.	NCT04260594, NCT04255017 and IRCT20180725040596N2
Enhancing the innate immune system	Natural killer cells (NK cells) [125]; play important role in cancer immunotherapy.	NCT04280224
	Recombinant interferon [126]; used as an antiviral or antineoplastic drug.	NCT04293887
	Mesenchymal stem cells (MSCs) [127]; found to be effective on acute lung injury (ALI)/acute respiratory distress syndrome (ARDS) occurred by both infectious and noninfectious diseases.	NCT04293692, NCT04269525, NCT04288102 and NCT04302519
Attenuating the inflammatory response	Intravenous immunoglobulin (IVIG) [128]; used to treat a number of health conditions.	NCT04261426
	Neutralizing antibodies (nAbs) [129]; used for anti-retroviral treatment.	Data not available
	Anti-C5a monoclonal antibody [130]; used for treating paroxysmal nocturnal hemoglobinuria and atypical hemolytic uremic syndrome (aHUS).	Data not available
	Blocking the interleukin (IL)-6 Pathway [131]; IL-6 inhibitors are approved for treatment of rheumatoid arthritis.	NCT04315298
	Thalidomide (Thalomid) [132]; used to treat or prevent Hansen's disease (leprosy). Also used in cancer treatment.	NCT04273529 and NCT04273581
	Methylprednisolone (Medrol) [133]; eases inflammation, used to treat arthritis and cancer.	NCT04273321 and NCT04263402
	Fingolimod (Gilenya) [134]; mostly used for treating multiple sclerosis.	NCT04280588
Symptomatic control	Bevacizumab (Avastin) [135]; used to treat cancer and eye disease.	NCT04275414
Vaccine	Lipid nanoparticle (LNP)-encapsulated mRNA [136]. mRNA-1273, a novel LNP-encapsulated mRNA-based vaccine, encoding full-length, prefusion stabilized spike (S) protein of SARS-CoV-2.	NCT04283461
	DNA vaccine (INO-4800) [137], being tested to prevent COVID-19 infection.	NCT04336410
	AZD1222 (ChAdOx1 nCoV-19) [138]; under clinical trials for COVID-19.	NCT04324606
	Nanoparticle-based vaccines [139]; candidate vaccines against various viral infections.	Data not available
Pathogen-specific artificial antigen-presenting cells	Clinical trials are evaluating the safety and immunogenicity of artificial antigen-presenting cells (aAPCs) alone and in combination with antigen-specific cytotoxic T cells [140]	NCT04299724 and NCT04276896

factor for the replication of CoV. Therefore, NSP13 has been recognized as a potential druggable target [95,96]. However, toxicity due to the non-specificity of inhibitors is considered to be the biggest hurdle [97]. On the basis of in silico studies drugs namely, lymecycline, cefsulodine, rolitetracycline, itraconazole and saquinavir were expected to be NTPase inhibitors [83].

Apart from the abovementioned drug targets, some NSPs that are critical players in viral RNA synthesis and replication, namely:

NSP3b, NSP3e, NSP6, NSP7-8 complex, NSP9, NSP10, NSP12 and NSP14-16, need further investigation for anti-viral drug discovery [60].

Targeting virulence factors

The SARS-CoV virulence factors that help the virus to escape the host immune system as well as interfere with the host's innate immunity are Nsp1, Nsp3c and ORF7. *(a)* Nsp1 inhibits type-I interferon production and is also responsible for degradation of the host mRNA [98,99]. *(b)* Nsp3c supports the in virus to resisting host innate immunity by binding with host's ADP-ribose [100]. *(c)* ORF7a binds and inhibits activity of the bone marrow matrix antigen 2 (BST-2) by blocking its glycosylation [54]. These effects of the virulence factors therefore advocate their potential for anti-viral drug advances.

Targeting host-specific receptors

Many studies have already unambiguously proved ACE2 as a receptor for S-RBD of coronavirus [101]. Recent work proves that the SARS-CoV-2 host receptor is constant among SARS-CoVs, therefore, the sequence of S-RBD in SARS-CoV-2 is similar, and central linking exists between the RBD receptor-binding motif and ACE2 receptors [102]. Therefore, ACE2 is considered as viable drug target for handling this infection. Arbidol (a broad-spectrum antiviral agent), which works against the influenza virus by inhibiting virus-host cell fusion and preventing virus entry into host cells, is under clinical trial for the treatment of SARS-CoV-2. Camostat mesylate, an existing TMPRSS2 (host cell protease, responsible for processing of spike protein and facilitating ACE2 binding) inhibitor, stops the entry of SARS-CoV-2 into host cells; thus, indicating its use as a prospective drug against this infection [93]. Despite several challenges and disagreements about targeting host receptors, several professional societies have recommended using this strategy for the treatment in COVID-19 patients.

Available regimens

Drug repurposing for SARS-Cov-2

To date, no medication has been approved for treating SARS-Cov-2 infection. Prevailing therapeutic options are not effective against this infection; however, a variety of possible treatments options are being explored by scientists [103]. In this situation the best way to tackle this infection will be drug repurposing. Considering the information obtained from genomic sequence along with in silico protein modelling, researchers have been working hard to find a way to defeat this menace.

Recently published work has recognized the interaction of SARS CoV-2 proteins with 332 human proteins. Out of these 332 protein-protein interactions, 66 were targetable by different antiviral compounds. All these compounds were further screened by multiple viral assays, resulting in the identification of two classes of antiviral compounds: *(i)* protein translation inhibitors (i.e., zotatifin, ternatin-4, and PS3061) and *(ii)* regulators Sigma1 and Sigma2 receptors (i.e., approved drugs: clemastine, cloperastine, progesterone and PB28) [60]. Some of these drugs have been reported to be more effective than hydroxychloroquine. In another study, researchers have screened about 12,000 FDA-approved drugs in clinical trials in Vero-E6 cells (African Green Monkey kidney) against SARS CoV-2 infection. Compounds, namely: apilimod (PIKfyve kinase inhibitor), cysteine protease inhibitors (MDL-28170, Z LVG CHN2, VBY-825, and ONO 5334), and a CCR1 antagonist (MLN-3897), were identified as potential druggable candidates against COVID-19 [104].

Severity of SARS CoV-2 infection is mostly among elderly patients, which is may be due to weak immune response due to the age factor. Therefore, adapting ways to boost innate immunity against viral attack shows great potential. A previous study has shown a promising role of macrophages and NK cells in the clearance of SARS-CoV after their pulmonary migration and thereby raising the levels of chemokines and cytokines [105]. There are several multinational companies who are utilizing this approach and aiming to reuse their NK-based products to combat COVID-19 infection. The most promising step has been taken by Cellularity (a USA-based company) by developing CYNK-001. Also, Type I interferons, used alone or in combination, give a broad-spectrum protection against viral infections including MERS-CoV [106] and SARS-CoV [107].

The SARS viral infection is majorly correllated with a high inflammatory response in the respiratory tract [108,109]. Hence, targeting mesenchymal stem cells (MSC) for therapeutic use in viral treatment has been proposed by various researchers, as these cells are acknowledged to exert anti-inflammatory responses and initiate the tissue repair mechanism [110]. The purpose of MSCs in treatment of COVID-19 pneumonia is still being investigated. Similarly, intravenous immunoglobulin (IVIG) is gaining attention for the treatment [111]. However, a more to the point approach for SARS-CoV treatment could be generation of surface specific epitope-targeting neutralizing antibodies [112]. Unfortunately, this is a time-consuming process and requires a lot of effort. The anti-C5a monoclonal antibodies may attenuate the degree of lung damage caused by COVID-19 by lowering the neutrophil influx and vascular leakage into the alveolar space [113]. When blocking the interleukin (IL)-6 pathway, as previously reported, a high level of IL-6 in blood rapidly reduces lung elasticity, resulting in acute bronchoalveolar inflammation. Thus, specific blocking of the IL-6-regulated signalling cascade may be considered as a valuable approach towards treatment [114]. The drug thalidomide which shows anti-inflammatory and anti-fibrotic effects, may reduce lung injury, and therefore it is also in the pipeline for COVID-19 patients. Moreover, clinical evaluation of methylprednisolone (a synthetic glucocorticoid) and fingolimod (an immunomodulating drug) for suppressing the undesired immune responces caused by SARS-CoV, is still in progress.

Every day knowledge about the COVID-19 virus is being updated and supporting the vaccine development process. However, that is a long-term stratagem to combat future outbreaks of SARS-CoV. There are various proposed vaccine candidates which are nucleic acid-based and in which the core revolves around the sequence-coding S protein. For instance, mRNA-1273 (Moderna) is developed to stimulate antiviral reaction, particularly against S-protein of COVID-19. Unlike traditional vaccines, development of this lipid nanoparticle (LNP)-encapsulated mRNA vaccine is free from any inactivated virus particle or subunit of live virus. The INO-4800 (Inovio Pharmaceuticals), is a genetic vaccine candidate that triggers immune response after being delivered to host cells. Additionally, these vaccines offer low cost and simple purification procedures as compared with traditional vaccines, and their simple structure prevents the chance of incorrect folding, which is a possibility in protein-based recombinant vaccines [106,110]. But the potency will depend upon the administration route and the numbers and intervals of plasmid doses delivered inside the body. Another candidate under evaluation is AZD1222/ChAdOx1 nCoV-19 (University of Oxford) constituting an adenovirus vector (non-replicating) carrying the COVID-19-S protein genetic sequence, making it comparatively safer in elderly individuals and children. The wide tissue (gastrointestinal and respiratory epithelium) range covered by adenovirus-based vectors increases their likelihood of forming an effective vaccine. However, the dominating immunogenicity for the vector will always be a concern [115].

Table 4
Patented vaccines against SARS-CoV and MERS-CoV [93].

Type of vaccine	Patent application	Target
Attenuated virus vaccines	US20060039926	The vaccine incorporates a mutation at specific tyrosine residue (Y6398H) into the viral genome encoding a p59 protein. Showing completely attenuated growth and pathogenicity of mouse coronavirus (MHV-A59).
DNA-based vaccines	WO2005081716	DNA vaccine targeting antigens of SARS-CoV, epitopes of the Membrane (M), Envelope (E), Spike (S) and Nucleocapsid (N) proteins of the virus. Stimulates immune responses (antigen-specific CD8+T cell mediated) against SARS-CoV antigens.
	WO2015081155	DNA-based vaccine comprised of MERS-CoV antigen (consensus spike protein).
Protein-based vaccines	WO2010063685	The vaccine comprises an immunogenic SARS spike protein and an adjuvant comprising an oil-in-water emulsion. Induces a protective immunity against the virus.
	US20070003577	The vaccine comprises purified trimeric S protein of SARS CoV, showing specific binding to ACE2 receptor.
	US20060002947	Ii-key/antigenic epitope hybrid peptide vaccines under clinical trials against COVID-19.
VIRUS-like particle vaccines	WO2015042373	The vaccine is composed of nanoparticles containing MERS virus proteins in polymer structures. Induces a neutralizing antibody response to MERS that reduces or prevents infection in mice and transgenic cattle.
mRNA-Based vaccines	WO2017070626	RNA vaccine and combination vaccine, composed of at least one mRNA encoding antigenic viral full-length S, S1, or S2 proteins from SARS-CoV and MERS-CoV virus, formulated in a cationic lipid nanoparticle.
	WO2018115527	mRNA based vaccine, encoding at least one antigen derived from a MERS-CoV and induces humoral immune responses.

Researchers are also putting effort into evaluating the efficacy of certain genetically modulated artificial antigen-presenting cells (aAPCs) specifically presenting the SARS-CoV structural proteins (conserved domains), and probably helping cells to endure the penetration of COVID-19 [116].

Moreover, there are several existing antiviral drugs which are under clinical trials for their potential against SARS-Cov-2 infection. Table 3 summarises the drugs that are in clinical trial which help in combating novel SARS-CoV2 infection.

Vaccines under development for COVID-19

Owing to the high morbidity and mortality associated with SARS-CoV-2 infection there is an urgent need for mitigation methods, and one of such method is vaccine development. Thorough research suggested that there is a significant sequence homology between SARS-CoV-2 and other beta-coronaviruses (SARS and MERS). The vaccines identified for these lethal coronaviruses can therefore be of high value in preparing vaccines against SARS-CoV-2 [141].

The viral S-protein based vaccine development approach has drawn the attention of many scientists in the fight against COVID-19. These subunit vaccines can elicit protective immunity, producing higher neutralizing antibodies as compared with DNA-based S protein vaccines, live-attenuated coronavirus and full-length S protein [142]. Presently, 188 well-established patents are present in the Chemical Abstracts Service (CAS) collection related to anti-SARS and anti-MERS vaccines. Most of them are associated with the S protein subunit vaccine and vaccines specifically targeting S-RBD [90]. Therefore, the preferred target for vaccine development against beta-coronaviruses is the S protein/gene, and applying the same strategy and knowledge will be beneficial in developing vaccines against SARS-CoV-2 [23,141]. Table 4 comprehends the list of SARS and MERS vaccines that have been patented.

Moreover, the prospect of short-term immunogenicity resulting from neutralising antibodies should be addressed. Along with B cell response, T cell response should also be considered, because these responses are protective and persistent in humans. Strategies for augmenting immunogenicity and preventing undesired side effects should be explored [141].

Summary

RNA viruses (SARS-CoV, MERS-CoV and SARS-CoV-2), causing severe mortal infections, will continue to be a serious global threat in future. These viruses have a high rate of mutation, genetic recombination and the capability of cross-species transmission, which make them a menace to mankind. The present outbreak of COVID-19 should direct us towards uplifting our knowledge and expertise in combating stubborn microbial pathogens and solving global health problems.

The present review summarizes recent research and developmental information published on international platforms related to SARS-CoV-2 infection, current therapeutic options and preventive vaccines. It includes a complete overview of the SARS-CoV-2 morphology, pathogenesis and antiviral strategies. Also, the drugs under clinical trials for COVID-19 have been discussed in detail, with the main focus on drug-repurposing which may be the best way out of this tragic situation. Literature strongly relates sequence similarity between SARS-CoV-2 and other beta-coronaviruses, and about 77.1% of the proteins found in SARS-CoV-2 are also reported in SARS CoV [143]. Mere ignorance of previous research on SARS and MERS proteins will increase vulgarity in drug and vaccine development for SARS-CoV-2. Therefore, utilizing the literature to forge a better understanding of SARS CoV-2 infection will enable us to design better antiviral drugs/vaccines against the virus. Furthermore, to speed up this drug development process, additional structural biological information including life-cycle details of the virus are required. At the same time, action should be taken to enhance SARS-CoV-2 surveillance systems, and individuals with symptoms (fever, cough or sore throat, diarrhea, body ache and rashes) should be screened for COVID-19.

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